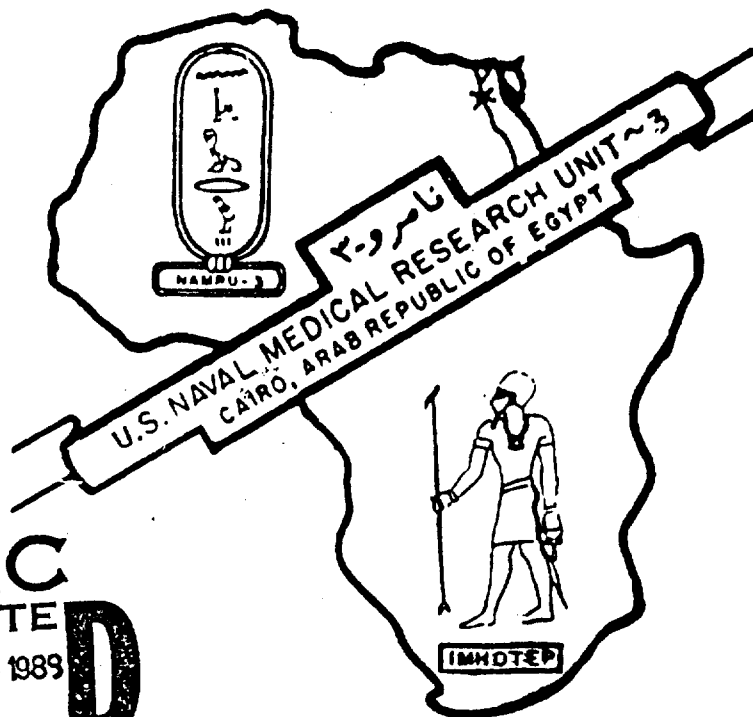


AD-A205 582



DTIC  
ELECTE  
MAR 13 1989  
D & D

# PUBLICATION REPORT

DISTRIBUTION STATEMENT A  
Approved for public release  
Distribution Unlimited

1493

12/88

STUDIES ON SOME DEVELOPMENTAL STAGES IN THE LIFE CYCLE OF  
PYGIDIOPSIS GENATA LOOSS. 1907 (TREMATODA: HETEROPHIDAE) FROM EGYPT

BY

Magda M. Youssef, Noshay S. Mansour, Nibal A. Hammouda,  
Helen N. Awadalla, Rifaat Khalifa and Laila M. Boulos

U.S. NAVAL MEDICAL RESEARCH UNIT NO. 3  
(CAIRO, ARAB REPUBLIC OF EGYPT)

FPO NEW YORK 09527

89 3 10 045

STUDIES ON SOME DEVELOPMENTAL STAGES IN  
THE LIFE CYCLE OF PYGIDIOPSIS GENATA Looss, 1907  
(Trematoda : Heterophidae) from Egypt\*

By

MAGDA M. YOUSSEF\*, NOSHY S. MANSOUR\*\*,  
NIBAL A. HAMMOUDA\*, HELEN N. AWADALLA\*,  
RIFAAT KHALIFA\*\*\* and LAILA M. BOULOS\*

Faculty of Medicine, Alexandria University\*,  
U.S. Naval Medical Research Unit No. 3, Cairo\*\*,  
and Faculty of Medicine, Assiut University\*\*\*, Egypt.

ABSTRACT

Pleurolophocercous cercariae of *Pygidiopsis genata* were obtained from naturally infected *Melania tuberculata* and allowed to encyst in clean *Gambusia affinis* and *Tilapia nilotica* under laboratory conditions. Metacercariae dissected from those fish

Request for reprints :

Research Publication Division  
NAMRU-3  
C/O American Embassy  
Cairo, Egypt.

- \* This work was partially supported by the Naval Medical Research and Development Command, NMC, NCR, Bethesda, Maryland, Work unit No. MR041.05.01.0053. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy or the naval service at large.

were fed to white mice. The juvenile stages of *P. genata* is described and the adult worm compared with the original description and related species.

## INTRODUCTION

*Pygidiopsis genata* (Looss 1907) was first described from a pelican (*Pelicanus onocrotalus*) in Egypt. The adult fluke is known to be a natural parasite of the small intestine of fish-eating birds (Looss 1907 ; Ciurea 1924 ; Gohar 1935) and mammals (Witenberg 1929 ; Abdel Azim 1938). Metacercariae of this parasite have been recorded in Egypt from *Mugil cephalus* (Fahmy and Selim 1959) and from *Tilapia* species (Boulos 1979) ; in Tunisia from *Astatotilapia desfontiansei* (Balozet and Callot 1939) ; in Palestine from *Barbus* species and *Tilapia simonis* (Witenberg 1929) ; in the Philippines from *Mugil* species (Vasquez-Colet and Africa 1938).

The present investigation describes the post miracidial and pre-adult stages in the life cycle.

## MATERIAL AND METHODS

*Melania tuberculata* (Bourg) were collected from Idku and Maryut lakes in Egypt and exposed to light about noon. Snails shedding pleurolophocercous cercariae were isolated as a source of cercariae and for dissection. Rediae and cercariae were studied alive, using 1% Nile blue sulphate as a vital stain, and in permanent preparations stained with Grenacher's borax carmine. From experimentally infected *Gambusia affinis* and *Tilapia nilotica*, metacercariae were dissected 4-6 weeks following exposure and fed to 6-week-old laboratory-reared white mice. Thereafter mice were sacrificed on days 1, 2 and 3 to recover juvenile worms from their intestines. These were studied alive or fixed in hot A.F.A., stained with Gowers carmine and mounted in permount. Measurements are in micrometers with ranges followed by mean in parentheses. Fifty of each stage were used for measurements and description. Figures were drawn with the aid of camera lucida.

## RESULTS

**Rediae (Fig. 1) :** The rediae occur in spaces within the digestive gland of the snail. They are sausage-shaped with a distinct constriction between middle and posterior third, and both ends rounded. Permanently stained specimens measured 480 to 700 (600) long and 90 to 130 (105) wide. The pharynx 25-30 long, well developed and followed by a short gut. Rediae are tightly packed with cercariae, ranging in development from small germ balls in the posterior portion to oculate cercariae anteriorly.

**Cercariae (Fig. 2,3) :** The natural infection rate of *Melania tuberculata* with *P. genata* was 0.98% (10 of 1024) in Idku lake and 1.6% (23 of 1436) in Maryut lake. In fresh preparations the emerged biocellate cercariae of *P. genata* swam in a rapid jerky manner with no characteristic resting position. They were strongly phototropic and emerged from the infected snails almost exclusively at noon. When stained with Nile blue sulphate, the 7 pairs of penetration glands, nuclei and granules of the body and tail, the genital primordium and tail fin became prominent. The latter begin dorsally near the body and extend posteriorly for about two-fifth of the tail length.

Stained, permanently mounted specimens : body ovoid, 108 to 140 (130) by 40 to 70 (60) ; body spines minute, decreasing in size and number posteriorly ; eye spots paired, quadrate, 7 to 8 by 6 to 7, about  $\frac{1}{3}$  body length from anterior end. Oral sucker subterminal, 23 to 26 (23) by 16 to 20 (18) ; ventral sucker indistinct, about  $\frac{2}{3}$  body length from anterior end ; prepharynx 18 ; pharynx indistinct between eye spots ; caeca not differentiated. Seven pairs of large unicellular penetration glands in middle third of body, 3 lateral and 4 median, opening around mouth in 3:4:4:3 pattern. Several cystogenous glands situated on both sides of penetration glands. Genital primordium medial, at level of ventral sucker. Excretory vesicle more or less Y-shaped, 15 to 22 (20) by 30 to 36 (32), the cavity lined with a single layer of cells. Tail set in shallow terminal groove, 385 to 420 by 13 to 17 (15),  $3\frac{1}{2}$  body length. Dorsoventral fin fold well differentiated in Nile blue vital stain.

Table 1 : Comparison between *Pygidiopsis genata* adults described by different authors (in micrometers)

	Looss (1907)	Ciurea (1924)	Witenberg (1929)	Present
Host	Pelican	Pelican	Dogs	Mice
Locality	Egypt	Roumania	Palestine	Egypt
Body length	300-500 (400)	580-780	400-700	350-560
Body breadth	200-220	250-340	200-400	160-270
Oral sucker	40 u with no oral spines	—	30-50 16 spines around the oral aperture seen only in fresh preparations	30-45 length 30-50 breadth
Ventral sucker	37-39 situated in the median plane	—	40-60	36 length 40 breadth to the right of the mediane plane
Prepharynx	long	—	30-100	30-45
Pharynx	36x24	—	20-40	36x33
Oesophagus	—	—	30-60	22-51
Bifurcation of the intestinal caeca	—	—	—	At a distance of 130-180 (160) from anterior end
Testes Right	—	—	60-140	50x69
Left	—	—	—	45x61
Ovary	—	—	40-80 globular	50x49 rounded
Seminal receptacle	—	—	70-140 globular	45-75
Eggs	21x11	—	13-22x9-12 with a conspi- cuous fila- ment at the posterior pole	17-22 (20) x 10-13 (11) no filament

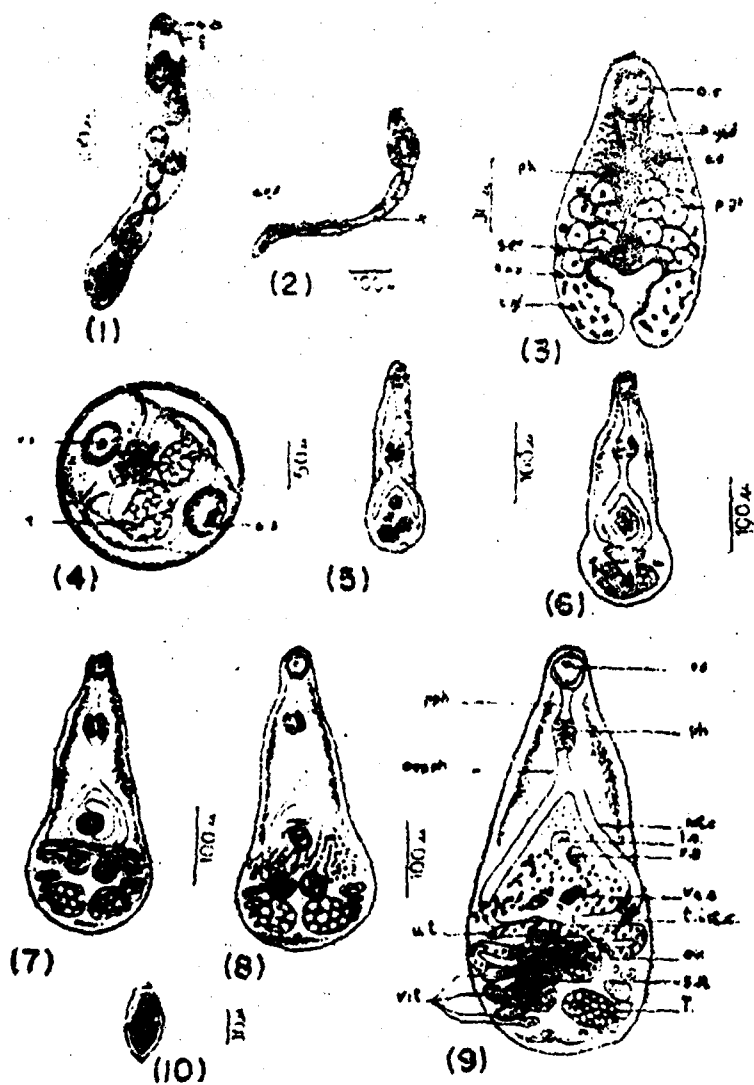
Table 2 : Comparison between *Pygidiopsis* species adults  
(in micrometers).

	pindoramensis Travassos, 1929	summa Onji et Nishio, 1916	phalacrocraciogenata Yamaguti, 1939	present-study
Body length	370-410	550-910	430-650	350-560
Body breadth	150-210	250-400	150-225	160-270
Oral sucker	subterminal 27	subterminal 30-54	ventro terminal 33-45	subterminal 30-45x30-50
Ventral sucker	44-48	43-60	33-45	36-40
Prepharynx	69-89	40-80	20-78	30-45
Pharynx	34-37x20-34	30-40x27-33	24-30x21-27	36x33
Oesophagus	24-44	75-150	60-105	22-51
Termination of intestinal caeca	At mid ovarian level	just in front of the testes	In front of the testes	In front of the testes
Testes	Symmetrical 34-51	(42-75) (75-120)	(54-60) (60-80)	Right 50x69 Left 45x61
Ovary	31-41	45-83	(42-60) (45-68)	(39-75) (30-60)
Seminal receptacle	—	50-70	—	45-75
Eggs	20x11	(21-23) (11-14)	(20-23) (11-14)	17-22 (20) 10-13 (11)

Metacercariae (Fig. 4,5) : Metacercariae were obtained mainly from head and tail regions of *Gambusia* and *Tilapia*. Viable encysted metacercariae exhibited a rotary movement inside double walled spherical or subspherical cysts 120 to 210 (186) in diameter. They are covered with scales and usually folded over. Oral and ventral suckers are well developed and the testes more prominent than are other genitalia. Pyriform body, 210 to 253 (236) by 51 to 85 (77), scaled except at posterior end. Oral sucker subterminal, 23 by 18 ; ventral sucker diameter 16 to 22. Prepharynx 50 to 60 long ; pharynx 27 to 30 long ; oesophagus 15 to 20 long, bifurcating postequatorially

midway between pharynx and ventral sucker; caeca curve around ventral sucker, terminating in anterior half of hindbody. Testes diagonal, 18 to 25 by 14 to 18. Excretory vesicle Y-shaped, easily seen in fresh preparations.

Figures 1-10 : Camera Lucida drawings of *P. genata*. 1 — Rediae ph. pharynx, g. gut. 2 — Cercaria t. tail, d.v.f. dorsoventral fin. 3. Body of cercaria. c. gl. cystogenous glands, e. s. eye spots, ex. v. excretory vesicle., g. pr. genital primordium, o. s. oral sucker, p. gl. d. penetration gland duct, ph. pharynx. 4. Encysted metacercaria, v. s. ventral sucker, T. testis. 5. Encysted metacercaria. 6. One day old juvenile worm. 7. Two days old juvenile worm. 8. Three days old juvenile worm. 9. Adult worm. int. c. intestinal caeca, l.o. lenticular organ, oesph. oesophagus, p. ph. pre-pharynx, s.r. seminal receptacle, t. int. c. termination of intestinal caeca, ut. uterus, ve. s. vesicula seminalis, vit. vitelline glands, 10-egg from stool of mouse.





Juvenile worms (Figs. 6, 7, 8) : Day-old worms (Fig. 6) : Body pyriform, 250 to 300 by 100 to 125. Prepharynx 45 ; oesophagus 27, bifurcated at midbody, caeca extend around ventral sucker into anterior half of hindbody, tips curving inward. Ovary well developed, pretesticular, median. Genital sac begins to appear anteroinferior to ventral sucker. Vitelline follicles, 3-4 in lateral fields.

Two-day old worms (Fig. 7) : Similar to above but larger (345x154), prepharynx shorter (27), oesophagus longer (45). Seminal receptacle clear, anterior and medial to left testis. Coiled uterus with few transparent eggs. Vitellaria more developed, forming large follicular masses. Ventrogenital sac more definite in outline and the ventral sucker distinct.

Three-day old worms (Fig. 8) : Nearly adult except for body size (381x172), few eggs in the uterus and vitelline follicles not yet fully developed.

Four-day old worms : Resemble adult except for body size, 392x180. Morphological and anatomical details of adult (Fig. 9) typical of *P. genata* as described by Looss (1907). Eggs ovoid (Fig. 10), transparent, light yellow shell, 17 to 22 (20) by 10 to 13 (11), with small rim at opercular junction.

#### DISCUSSION

Several pleurolophocercous cercariae have been reported from Egypt by various authors (Sonsino 1892 ; Looss 1896 ; Khalil 1932 ; El Guindy and Hanna 1963 ; Fahmy et al. 1976 ; Khalifa et al. 1977). The one described by Looss (1896) after Sonsino, plate XIII Fig. 143, is probably the larva of *P. genata* and is similar to the present cercarial description. Encysted metacercariae were frequently found in the muscles of head and tail regions of the experimentally infected fish, as is found in naturally infected ones (Boulos 1979). The encysted metacercariae of *P. genata* and the 1, 2, 3, and 4-day juveniles from experimental infection in white mice are described here for the first time. Some organs of the genital system such as ovary

and vitellaria, can be recognized by the first day, while few uterine eggs are seen before the third day, contrary to *Pygidioopsis summa* (Onji and Nishio 1916) in which well developed worms were recovered as early as 5 hours after feeding to white rats. Even with this species, however, egg-producing maturity was attained in about a week and full maturity in 2 weeks (Ochi 1931).

Differences in measurements and in certain internal structures have been found among *P. genata* adults described by different workers (Table 1). Measurements in this study agree with those of Looss (1907), and are smaller than those of Ciurea (1924) and Witenberg (1929). Sixteen spines were found around the oral sucker by Witenberg (1929), who observed them in fresh specimens. These could not be seen in the present material and were not mentioned in the other descriptions (Looss 1907; Ciurea 1924). In the present study, bifurcation of the intestinal caeca occurs 130 to 180 from the anterior end, an observation not reported elsewhere. The short intestinal caeca, terminating above the testes, is common to all descriptions. The testes are transversely oval, the right testis being slightly larger. This is not mentioned in other descriptions but is depicted in Fig. 22 by Witenberg (1929). The ventral sucker, well developed and included in the ventrogenital sac, is here similar to that described by Looss (1907) but smaller than that described by Witenberg (1929). The latter authors reported the ventral sucker to be median, whereas Ciurea (1924) found the center of the ventral sucker displaced slightly to the right, in agreement with present material. Size differences can be attributed to degree of development in different host species or perhaps to differences in methods of preparation.

*P. genata* egg size in Looss (1907) and Witenberg (1929) agrees with those in the present study.

Major morphological and egg size similarities support the view that the parasite in the present study is *P. genata*, showing identity with the same species described in various hosts and localities by Looss (1907), Witenberg (1929) and Ciurea (1924).

*Pygidiopsis genata* as described here differs from other species of *Pygidiopsis* (Table 2). *P. pindoramensis* (Travassos 1929) has a small depression in the median plane, characterizing this species; it is smaller but with a longer pharynx, and intestinal caeca that terminate at a lower level than in *P. genata*. Minute spines cover the entire body, including the posterior portion, which is devoid of them in *P. genata*. The genital pore is submedian, opening immediately below the acetabulum, whereas it opens above it in *P. genata*. *P. summa* (Yamaguti 1939) and *P. phalacrocracis* (Yamaguti 1939) are larger with longer intestinal caeca (reaching the level of the testes) than is found in *P. genata*.

#### REFERENCES

- Abdel Azim, M. (1938) : On the intestinal helminths of dogs in Egypt. J. Egypt. Med. Assoc, Cairo 21:1-5.
- Balozet, L., Callot, J. (1939) : Trematodes de Tunisie, 3. Super-famille Heterophyoidea. Archives de l'Institut Pasteur de Tunis 28:34-63.
- Boulos, L.M. (1979) : Parasitological studies on the heterophyids in *Tilapia* from Idku and Maryut Lakes and their transmission to laboratory animals. M.D. thesis, Alexandria University.
- Ciurea, J. (1924) : Heterophyides de la faune parasitaire de Roumanie. Parasitol. 16:1-21.
- El-Guindy, M.S., Hanna, F.Y. (1963) : Larval trematodes from snails *Pirenella conica* and *Melania tuberculata* with special reference to heterophyiasis. Bull. End. Dis., Baghdad, 5:33-58.
- Fahmy, M.A.M., Mandour, A.M., Arafa, A.S., Omran, L.A.M. (1976) : Larval trematodes recovered from *Melania tuberculata* in Assiut Province, Assiut Vet. Med. J. 3: 241-250.
- Fahmy, M.A.M., Selim, M.K. (1959) : Studies on some trematode parasites of dogs in Egypt with special reference to the role played by fish in their transmission. Zeitschrift für Parasitenkunde 19:3-13.
- Gohar, N. (1935) : Liste des trematodes parasites et de leur

notes vertebres signales dans la Vallee du Nil. Deuxième partie. Ann. Parasitol. Humaine et Comparee 13:80-90.

- Khalifa, R., El Naffar, M.K., Arafa, M.S. (1977) : Studies on heterophyid cercariae from Assiut Province. 1 — Notes of the life cycle of *Haplorchis pumilio* (Looss 1896) (Trematoda, Heterophyidae) with a discussion on previously described forms. Acta Parasitol. Polonica 25: 325-38.
- Khalil, M. (1932) : The life history of a heterophyid parasite in Egypt. Compte rendu. Congr. Inter. Med. Trop. et Hyg. 4:137-147.
- Looss, A. (1896) : Recherches sur la faune parasitaire de l'Egypte. Première partie. Mémoires de l'Institut Egyptien. Le Caire 3:1-252.
- Looss, A. (1907) : Notizen sur Helminthologie Aegyptens II. Über einige neue Trematoden der Agyptischen Fauna. Zentralblatt für Bakteriologie, Parasitenkunde Infektionskrankheiten und Hygiene 43:478-490.
- Ochi, S. (1931) : Studies on trematodes with brackish water, fishes and intermediate hosts. On the life history of *Pygidiopsis summa*. Tokyo Iji Shinshi 2712:346-353 ; summarized by Yamaguti S (1975). A synoptical review of life histories of digenetic trematodes of vertebrates with special reference to the Morphology of their larval forms (Kyoto, Japan).
- Onji, Y., Nishio, K. (1916) : A review of new intestinal flukes. Igaku Chuo Zasshi 14:439-442. Quoted from Pearson JC (1964). A revision of the subfamily Haplorchinae Trematode Heterophidae. Parasitol. 54:601-676.
- Sonsino, P. (1892) : Studie sui parassiti de molluschi di acqua dolce nei dintorni di Cairo in Egitto. Festschrift 70 Geburtstag. R. Leucharts 134-146.
- Travassos (1929) : Sur une nouvelle espece du genre *Pygidiopsis* *pindoramensis* n. sp. Trematoda C R Soc Biol 100: 956-957.

Vasquez-Colet, A., Africa, C.M. (1938) : Determination of the piscine intermediate hosts of Philippine heterophyid trematodes by feeding experiments. Philippine J. Sc. 65:293-302.

Witenberg, G. (1929) : Studies on the trematodes family Heterophyidae. Ann. Trop. Med. Parasit. 23:131-239.

Yamaguti, S. (1939) : Studies on the helminth fauna of Japan. Part. 25. Trematodes of birds IV. Jpn. J. Zool. 8:129-210.

Accession For	
NTIS CRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and/or Special
A-1	20



UNCLASSIFIED  
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE				
1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED		1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; Distribution is unlimited		
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE				
4. PERFORMING ORGANIZATION REPORT NUMBER(S) 12/88		5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION U.S. Naval Medical Research Unit No. 3		6b. OFFICE SYMBOL (If applicable) NAVMEDRSCHU THREE		7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) FPO New York 09527-1600		7b. ADDRESS (City, State, and ZIP Code)		
8a. NAME OF FUNDING / SPONSORING ORGANIZATION Naval Medical Research and Development Command		8b. OFFICE SYMBOL (If applicable) NAVMEDRSCHDEV COM		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) Naval Medical Command National Capital Region Bethesda, MD 20814-5044		10. SOURCE OF FUNDING NUMBERS		
		PROGRAM ELEMENT NO. 61152N	PROJECT NO MR041- 05	TASK NO 01
11. TITLE (Include Security Classification) Studies on Some Developmental Stages in the Life Cycle of <u>Pygidiopsis genata</u> Looss. 1907 (Trematoda: Heterophidae) from Egypt. (UNCLASSIFIED)				
12. PERSONAL AUTHOR(S) Youssef, Magda M.*, Mansour, Noshay S., Hammouda, Nibal A.*, Awadalla, Helen N.*, Khalifa, Rifaat** and Boulos, Laila M.*				
13a. TYPE OF REPORT	13b. TIME COVERED FROM TO		14. DATE OF REPORT (Year, Month, Day) 1987	15. PAGE COUNT 12
16. SUPPLEMENTARY NOTATION Published in: J. Egypt. Soc. Parasitol., 17(2):463-474, 1987; Acc. No. 1493.				
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)  <u>Pygidiopsis genata</u> ; Life cycle; Egypt	
FIELD	GROUP	SUB-GROUP		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) Pleurolophocercous cercariae of <u>Pygidiopsis genata</u> were obtained from naturally infected <u>Melania tuberculata</u> and allowed to encyst in clean <u>Gambusia affinis</u> and <u>Tilapia nilotica</u> under laboratory conditions. Metacercariae dissected from those fish were fed to white mice. The juvenile stages of <u>P. genata</u> is described and the adult worm compared with the original description and related species. <i>Keywords: Trematodes, Medical Research, Naval Research, Reprints, C.A.W.</i> * Faculty of Medicine, Alexandria University, Alexandria, Egypt. ** Faculty of Medicine, Assiut University, Assiut, Egypt.				
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL Research Publication Branch			22b. TELEPHONE (Include Area Code) 820727 (Cairo, Egypt)	22c. OFFICE SYMBOL R.P.B.

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted  
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE

U.S. Government Printing Office: 1980-607-044

UNCLASSIFIED